CLAIMS

1. A sucrose phosphorylase having improved thermostability, which is obtained by modifying a natural sucrose phosphorylase,

wherein the sucrose phosphorylase having improved thermostability has an amino acid residue which is different from that of the natural sucrose phosphorylase in at least one position selected from the group consisting of:

a position corresponding to position 14, a position corresponding to position 29 and a position corresponding to position 44 in motif sequence 1: AVGGVHLLPFFPSTGDRGFAPIDYHEVDSAFGDWDDVKRLGEKYYLMFDFMINHIS;

a position corresponding to position 7, a position corresponding to position 19, a position corresponding to position 23 and a position corresponding to position 34 in motif sequence 2: RPTQEDVDLIYKRKDRAPKQEIQFADGSVEHLWNTFGEEQID; and

a position corresponding to position 19 in motif sequence 3: ILPEIHEHYTIQFKIADHDYYVYDFALPMVTLYSLYSG; and

wherein enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C, after heating the sucrose phosphorylase having improved thermostability in 20 mM Tris buffer (pH 7.0) at 55°C for 20 minutes, is 20% or more of enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C, before heating.

2. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid sequence of the natural sucrose phosphorylase has at least 40% identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO:

6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18 and SEQ ID NO: 20.

3. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid sequence of the natural sucrose phosphorylase has at least 60% identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18 and SEQ ID NO: 20.

4. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid sequence of the natural sucrose phosphorylase is encoded by a nucleic acid molecule which hybridizes under stringent conditions to a nucleic acid molecule consisting of a base sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18 and SEQ ID NO: 20.

5. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid sequence of the natural sucrose phosphorylase is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18 and SEQ ID NO: 20.

6. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the natural sucrose phosphorylase is derived from a bacterium selected from the group consisting of *Streptococcus mutans*,

Streptococcus pneumoniae, *Streptococcus sorbinus*, *Streptococcus mitis*, *Leuconostoc mesenteroides*, *Oenococcus oeni*, *Lactobacillus acidophilus* and *Listeria monocytogenes*.

7. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the natural sucrose phosphorylase is derived from *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus sorbinus* or *Streptococcus mitis*.

8. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the natural sucrose phosphorylase is derived from *Streptococcus mutans* or *Leuconostoc mesenteroides*.

9. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid residue in a position corresponding to position 14 in motif sequence 1 is serine or isoleucine.

10. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid residue in a position corresponding to position 29 in motif sequence 1 is proline, alanine or lysine.

11. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid residue in a position corresponding to position 44 in motif sequence 1 is histidine, arginine or tryptophan.

12. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid residue in a position corresponding to position 44 in motif

sequence 1 is arginine.

13. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid residue in a position corresponding to position 7 in motif sequence 2 is leucine or isoleucine.

14. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid residue in a position corresponding to position 19 in motif sequence 2 is methionine, cysteine, phenylalanine, isoleucine, valine or tyrosine.

15. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid residue in a position corresponding to position 19 in motif sequence 2 is valine or tyrosine.

16. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid residue in a position corresponding to position 23 in motif sequence 2 is arginine, histidine, isoleucine, lysine or valine.

17. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid residue in a position corresponding to position 23 in motif sequence 2 is histidine.

18. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid residue in a position corresponding to position 34 in motif sequence 2 is serine or threonine.

19. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid residue in a position corresponding to position 34 in motif sequence 2 is serine.

20. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid residue in a position corresponding to position 19 in motif sequence 3 is glycine, cysteine, histidine, lysine, leucine, asparagine, proline, glutamine, arginine or serine.

21. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the amino acid residue in a position corresponding to position 19 in motif sequence 3 is glycine.

22. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C, after heating the sucrose phosphorylase having improved thermostability in 20 mM Tris buffer (pH 7.0) at 57°C for 20 minutes is 10% or more of the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C before heating.

23. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C, after heating the sucrose phosphorylase having improved thermostability in 20 mM Tris buffer (pH 7.0) at 57°C for 20 minutes, is 20% or more of the enzyme activity of the sucrose phosphorylase having

improved thermostability at 37°C before heating.

24. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C, after heating the sucrose phosphorylase having improved thermostability in 20 mM Tris buffer (pH 7.0) containing 20% sucrose at 65°C for 20 minutes is 10% or more of the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C before heating.

25. The sucrose phosphorylase having improved thermostability according to claim 1, wherein the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C, after heating the sucrose phosphorylase having improved thermostability in 20 mM Tris buffer (pH 7.0) containing 20% sucrose at 65°C for 20 minutes, is 20% or more of the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C before heating.

26. The sucrose phosphorylase having improved thermostability according to claim 1, which has an amino acid residue which is different from that of the natural sucrose phosphorylase in at least a position corresponding to position 14 in motif sequence 1.

27. The sucrose phosphorylase having improved thermostability according to claim 1, which has an amino acid residue which is different from that of the natural sucrose phosphorylase in at least a position corresponding to position 19 in motif sequence 3.

28. A method for producing sucrose phosphorylase having improved thermostability, comprising:

modifying a first nucleic acid molecule comprising a base sequence encoding a first sucrose phosphorylase to obtain a second nucleic acid molecule comprising a modified base sequence;

preparing an expression vector containing the second nucleic acid molecule;

introducing the expression vector into a cell to express sucrose phosphorylase having improved thermostability; and

recovering the expressed sucrose phosphorylase having improved thermostability,

wherein the sucrose phosphorylase having improved thermostability has an amino acid residue which is different from the amino acid residue of the first sucrose phosphorylase in at least one position selected from the group consisting of:

a position corresponding to position 14, a position corresponding to position 29 and a position corresponding to position 44 in motif sequence 1:
AVGGVHLLPFFPSTGDRGFAPIDYHEVDSAFGDWDDVKRLGEKYYLMFDFMINHI
S;

a position corresponding to position 7, a position corresponding to position 19, a position corresponding to position 23 and a position corresponding to position 34 in motif sequence 2:
RPTQEDVDLIYKRKDRAPKQEIQFADGSVEHLWNTFGEEQID; and

a position corresponding to position 19 in motif sequence 3: ILPEIHEHYTIQFKIADHDYYVYDFALPMVTLYSLYSG; and

wherein the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C after heating the sucrose phosphorylase having improved thermostability in

20 mM Tris buffer (pH 7.0) at 55°C for 20 minutes, is 20% or more of the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C before heating.

29. The method according to claim 28, wherein the amino acid sequence of the first sucrose phosphorylase has at least 40% identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18 and SEQ ID NO: 20.

30. The method according to claim 28, wherein the amino acid sequence of the first sucrose phosphorylase has at least 60% identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18 and SEQ ID NO: 20.

31. The method according to claim 28, wherein the amino acid sequence of the first sucrose phosphorylase is encoded by a nucleic acid molecule hybridizing under stringent conditions with a nucleic acid molecule consisting of a base sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18 and SEQ ID NO: 20.

32. The method according to claim 28, wherein the amino acid sequence of the first sucrose phosphorylase is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18 and SEQ ID NO: 20.

33. The method according to claim 28, wherein the first sucrose phosphorylase is derived from a bacterium selected from the group consisting of *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus sorbinus*, *Streptococcus mitis*, *Leuconostoc mesenteroides*, *Oenococcus oeni*, *Lactobacillus acidophilus* and *Listeria monocytogenes*.

34. The method according to claim 28, wherein the first sucrose phosphorylase is derived from *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus sorbinus* or *Streptococcus mitis*.

35. The method according to claim 28, wherein the natural sucrose phosphorylase is derived from *Streptococcus mutans* or *Leuconostoc mesenteroides*.

36. The method according to claim 28, wherein the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C, after heating the sucrose phosphorylase having improved thermostability in 20 mM Tris buffer (pH 7.0) at 57°C for 20 minutes is 10% or more of the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C before heating.

37. The method according to claim 28, wherein the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C, after heating the sucrose phosphorylase having improved thermostability in 20 mM Tris buffer (pH 7.0) at 57°C for 20 minutes, is 20% or more of the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C before heating.

38. The method according to claim 28, wherein the sucrose

phosphorylase having improved thermostability has an amino acid residue which is different from that of the first sucrose phosphorylase in at least a position corresponding to position 14 in motif sequence 1.

39. The method according to claim 28, wherein the sucrose phosphorylase having improved thermostability has an amino acid residue which is different from that of the first sucrose phosphorylase in at least a position corresponding to position 19 in motif sequence 3.

40. A nucleic acid molecule comprising a base sequence encoding the sucrose phosphorylase having improved thermostability according to claim 1.

41. A vector comprising the nucleic acid molecule according to claim 40.

42. A cell comprising the nucleic acid molecule according to claim 40.

43. A method of synthesizing glucose-1-phosphate, comprising reacting a reaction solution containing the sucrose phosphorylase having improved thermostability according to claim 1, sucrose and inorganic phosphoric acid to produce glucose-1-phosphate.

44. The method according to claim 43, wherein the reaction is carried out at a temperature of 50°C to 70°C.

45. A method of synthesizing a glucose polymer, comprising reacting a reaction solution containing the sucrose phosphorylase having improved thermostability according to

claim 1; a second phosphorylase using α-glucose-1-phosphate as a substrate; sucrose; a primer; and inorganic phosphoric acid or glucose-1-phosphate to produce a glucose polymer.

46. The method according to claim 45, wherein the glucose polymer is an α-glucan.

47. The method according to claim 45, wherein the second phosphorylase is an α-glucan phosphorylase.

48. The method according to claim 45, wherein the second phosphorylase is selected from the group consisting of cellobiose phosphorylase, cellodextrin phosphorylase, laminaribiose phosphorylase, laminaridextrin phosphorylase, β-1,3-glucan phosphorylase and trehalose phosphorylase.

49. The method according to claim 45, wherein the reaction is carried out at a temperature of 50°C to 70°C.

50. A sucrose phosphorylase having improved thermostability, obtained by modifying a natural sucrose phosphorylase,

wherein the sucrose phosphorylase having improved thermostability has an amino acid residue which is different from that of the natural sucrose phosphorylase in at least one position selected from the group consisting of:
a position corresponding to threonine at position 47 (T47);
a position corresponding to serine at position 62 (S62);
a position corresponding to tyrosine at position 77 (Y77);
a position corresponding to valine at position 128 (V128);
a position corresponding to lysine at position 140 (K140);
a position corresponding to glutamine at position 144 (Q144);
a position corresponding to asparagine at position 155 (N155); and a position corresponding to aspartic acid at

position 249 (D249);
in the amino acid sequence of SEQ ID NO: 2; and

wherein the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C, after heating the sucrose phosphorylase having improved thermostability in 20 mM Tris buffer (pH 7.0) at 55°C for 20 minutes, is 20% or more of the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C before heating.

51. A method for producing sucrose phosphorylase having improved thermostability comprising:

modifying a first nucleic acid molecule comprising a base sequence encoding a first sucrose phosphorylase to obtain a second nucleic acid molecule comprising a modified base sequence;

preparing an expression vector containing the second nucleic acid molecule;

introducing the expression vector into a cell to express sucrose phosphorylase having improved thermostability; and

recovering the expressed sucrose phosphorylase having improved thermostability,

wherein the sucrose phosphorylase having improved thermostability has an amino acid residue which is different from the amino acid residue of the first sucrose phosphorylase, in at least one position selected from the group consisting of:
a position corresponding to threonine at position 47 (T47);
a position corresponding to serine at position 62 (S62);
a position corresponding to tyrosine at position 77 (Y77);
a position corresponding to valine at position 128 (V128);
a position corresponding to lysine at position 140 (K140);
a position corresponding to glutamine at position 144 (Q144);
a position corresponding to asparagine at position 155

(N155); and a position corresponding to aspartic acid at position 249 (D249);
in the amino acid sequence of SEQ ID NO: 2; and

wherein the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C, after heating the sucrose phosphorylase having improved thermostability in 20 mM Tris buffer (pH 7.0) at 55°C for 20 minutes, is 20% or more of the enzyme activity of the sucrose phosphorylase having improved thermostability at 37°C before heating.

52. A method of synthesizing glucose-1-phosphate, comprising reacting a reaction solution containing the sucrose phosphorylase having improved thermostability according to claim 50, sucrose and inorganic phosphoric acid to produce glucose-1-phosphate.

53. A method of synthesizing a glucose polymer comprising reacting a reaction solution containing: the sucrose phosphorylase having improved thermostability according to claim 50; a second phosphorylase using α-glucose-1-phosphate as a substrate; sucrose; a primer; and inorganic phosphoric acid or glucose-1-phosphate; to produce a glucose polymer.